

# Gas Chromatographic Study of the Steam Volatile Fatty Acids of Various Tobaccos

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Past studies from this laboratory have concerned the qualitative or quantitative composition of leaf sterols (1, 2), aliphatic paraffins (3), neophytadiene (4), solanesol-like substances (5), and higher fatty acids (6) in different tobaccos. Recently, the volatile neutral components of cigarette tobaccos (7) and cigarette smoke (8) have been investigated. In a continuation of this work, the comparative composition of the steam-volatile fatty acids in cigarette tobaccos has now been studied by gas chromatography and greater differences have been found than in the previous investigations cited above. The findings should be considered preliminary in nature.

Since the formation of derivatives of the lower fatty acids (or related techniques (9)) for subsequent gas chromatographic separation possess certain shortcomings (e.g. low yields (10), artifacts from reagents (9), etc.), efforts were limited to the separation of the free acids. Several stationary phases have been reported as suitable for such separations, including silicone-stearic acid (11), Tween\* 80 (12, 13) or diethylene glycol adipate polyester (14) (DEGA) with or without phosphoric acid, and "Trimer Acid" (15). In the present work, two stationary phases were employed (25% Tween 80-2% phosphoric acid on gas Chrom P or 25% DEGA-2% phosphoric acid on Chromosorb W) in either a single column (2 ft. x 0.25 in.), thermal conductivity detection system or dual column (5 ft. x 0.125 in.), flame ionization system. The Tween column was operated isothermally at 110°, and the DEGA column was run at 125° isothermally or programmed at 6°/minute from 100 to 211°. For identification, co-

chromatography of the unknowns with authentic compounds was performed on the two columns, paper chromatographic separations of the acids were made by the methods of Schwartzman (16), Isherwood and Hanes (17), and Reid and Lederer (18), and infrared spectra were obtained on collected gas chromatographic fractions.

The tobacco samples and the methods of steam-distillation and isolation of the acidic substances, including limitations thereof, have been previously described (7). The acidic substances are ultimately obtained in a concentrated ether solution for gas chromatographic study. Although the removal of solvent during the concentration step has been shown to give significant losses of methyl esters of fatty acids (19), comparable losses were not observed for the free fatty acids under these conditions: recoveries of *n*- and *iso*-acids ( $C_1$ - $C_8$ ) were greater than 87% except for isovaleric (83%) and caprylic (75%). It should be noted that the method does not measure total (free and bound), steam-volatile acids but is simply a relative measure of the volatile acids which steam-distill at the pH of the tobacco under the conditions used.

Comparisons of the four major types of cigarette tobaccos (bright, burley, Maryland, and Turkish) revealed major differences in the amounts and distribution of lower fatty acids. In the various tobaccos, formic, acetic, propionic, isobutyric, *n*-butyric, isovaleric, *n*-valeric,  $\beta$ -methylvaleric, isocaproic, *n*-caproic, *n*-heptylic, and *n*-caprylic were found. Except for *n*-butyric, *n*-heptylic, and isocaproic, the occurrence of these acids in tobacco leaf has been previously known; *n*-butyric,

*n*-heptylic, and *n*-caprylic have been reported in tobacco smoke (20, 21). In general, burley and Maryland tobaccos contained relatively small amounts of the acids with Maryland showing more than burley. Turkish (Samsun) and bright tobaccos contained relatively large amounts of the acids with distinct differences between the two types (Figs. 1-3). Turkish (Samsun) showed much larger amounts (more than fourfold) of  $\beta$ -methylvaleric and smaller amounts (less than one-half) of *n*-valeric acid compared to most samples of bright. An unidentified peak (9, Fig. 1) eluting between isocaproic and caproic was present in bright and absent in Turkish (Samsun). Turkish (Smyrna) was similar to Turkish (Samsun) except that smaller amounts of all acids were apparent.

Differences were also observed between "aromatic" and "aroma-deficient" grades of bright tobacco (Figs. 1-2) although the variations were slighter than in the above comparison. In general, aromatic grades showed larger amounts of isobutyric, *n*-valeric, isovaleric, and  $\beta$ -methylvaleric acids than aroma-deficient tobaccos but the differences in the case of the last three acids were small and possibly of questionable significance. Aroma-deficient tobacco also gave a component which eluted as an inflection on unidentified peak 9 and was absent in the aromatic samples.

The overall method appears to be rapid and reliable for comparative studies on natural products of a fundamentally similar nature. Using optimal chromatographic conditions

\*Mention of a specific commercial product does not constitute endorsement by the U.S. Department of Agriculture.

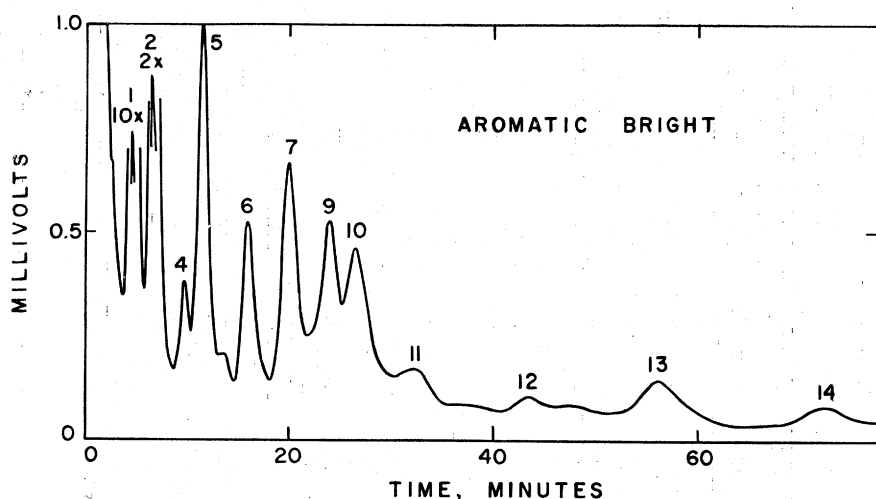


Figure 1. Separation of volatile acids from bright tobacco (aromatic grade). Chromatographic conditions: dual columns (5 ft. x 0.125 in.) containing 25% Tween 80-2% phosphoric acid on Gas Chrom P; column temperature, 110°; carrier gas (helium) flow, 40 ml. per min.; dual flame ionization detectors (hydrogen pressure, 20 psi; air pressure, 38 psi). Peak identities (all acids): 1- acetic, 2- propionic and formic, 4- *n*-butyric, 5- isovaleric, 6- *n*-valeric, 7-  $\beta$ -methylvaleric, 10- *n*-caproic, 12- *n*-heptylic, 14- *n*-caprylic. Peaks 9, 11, and 13 are unidentified.

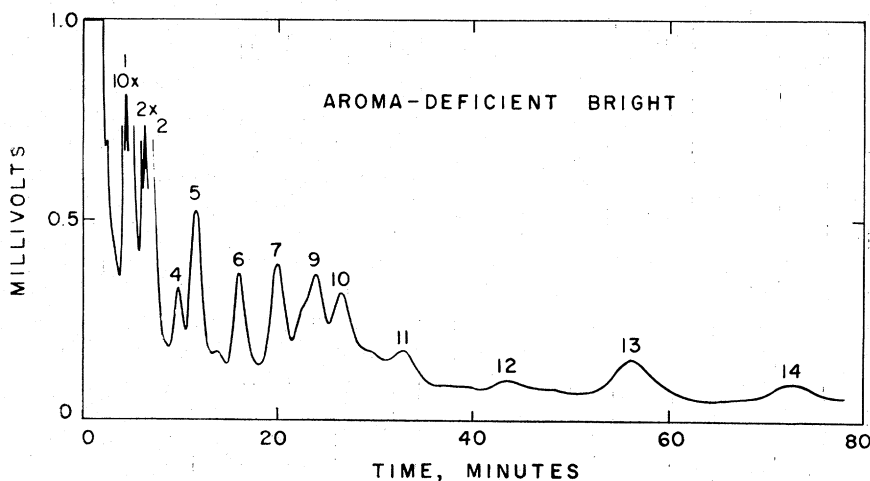


Figure 2. Separation of volatile acids from bright tobacco (aroma-deficient grade). See Figures 1 and 3 for conditions and peak identities.

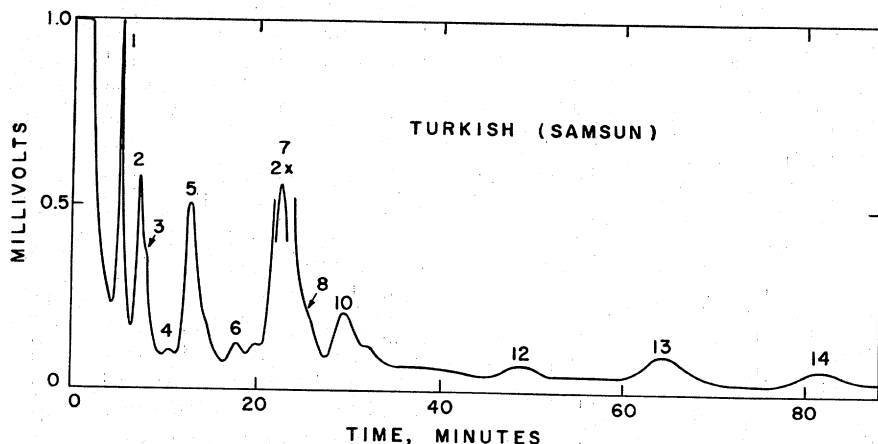


Figure 3. Separation of volatile acids from Turkish (Samsun) tobacco, See Figure 1 for chromatographic conditions and peak identities. Peak 3- isobutyric acid, peak 8- isocaproic acid.

with both columns, satisfactory resolution is obtained for the lower *n*- and *iso*- acids although formic and propionic do not separate on the Tween column when either acid is in great excess. In general, peaks are sharper and resolution of certain acids (propionic from isobutyric,  $\beta$ -methylvaleric from isocaproic) is superior on the DEGA column, although *C*<sub>1</sub> and *C*<sub>2</sub> are eluted simultaneously.

Further details of this study will be published at another time. The authors acknowledge the assistance of Dr. D. Burdick, Mr. R. L. Chrzanowski and Mrs. W. R. Bilinsky in this investigation. ■

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